UDC 581.19 : 582.23

Photochemical Reaction of Ubiquinone (35). I. Formation of Demethyl-ubiquinone (35).

In the course of investigation on the stability of ubiquinone (35) (referred to as UQ (35) hereinafter) the author observed that UQ (35) was converted by irradiation with sunlight into several substances some of which had a biological activity similar to that of UQ (35). The author has clarified the chemical structure of one of the substances. The material used was UQ (35) obtained from the mycelium of *Torula utilis* and its solution in ethanol was irradiated with sunlight of 0.35~0.7 million luxes per hour. A small quantity of the reaction mixture was chromatographed[1] with propanol-water (4:1) on filter paper (Whatman No. 1) impregnated with silicone, and the resulting chromatogram was investigated using 0.2% potassium permanganate solution as coloring agent to give at least five spots other than that of UQ (35). The reaction mixture was then adsorbed on a column of silicic acid-Hyflo Super-Cel (diatomaceous earth) (2:1), when the column was colored stratum-wise to give yellow, orange, violet and brown bands. Each band of the chromatogram thus resulted was eluted successively with mixtures of hexane and chloroform in various proportions to furnish fractions (A~I).

Table I. The Rf-Value of Each Fraction Separated on Silicic Acid Column Chromatography of Reaction Mixture

Fraction name	Rf[a]								
A			0.65						
B	0.47								
C	(0.47)					0.78			
D		0.56							
E		(0.56)			0.73				
F			0.65	(0.71)					
G				0.71			(0·86)		
H							0.86		
I									0.92

a) Rf-value of UQ(35) is 0.47.
Figures bracketed are Rf-values of by-products.
Solvent system : PrOH-H_2O (4:1)
Paper : Whatman No. 1 impregnated with silicone
Ascending method

The Rf-values of the fractions are as shown in Table I.

The fraction H was again adsorbed on a column of Florisil (magnesium silicate, 60~100 mesh) and, after removing impurities by washing the column with hexane-ethanol (1:1), a pure substance (tentatively called Substance (I) was eluted with methanol and crystallized as orange red plates from MeOH, m.p. 38°,*1 Rf 0.86 (the method was the same as shown in Table I). *Anal.* Calcd. for $C_{43}H_{64}O_4$: C, 80.12; H, 9.95; 1 OMe, 4.81; mol. wt., 644. Found : C, 79.71; H, 10.02; OMe (Zeisel), 4.75; mol. wt. (Barger), 626±25. Oxidized form, UV : λ_{max}^{EtOH} 275 mμ ($E_{1cm}^{1\%}$ 133), λ_{min}^{EtOH} 240 mμ ($E_{1cm}^{1\%}$ 39); reduced form, UV : λ_{max}^{EtOH} 350 mμ ($E_{1cm}^{1\%}$ 51)→295 mμ (37). IR ν_{max}^{Liquid} cm^{-1} : 3350 (OH); 1665, 1610 (quinone); 1258, 1207, 1150, 1093 (OMe); NMR (Table II).

Substance (I) is positive to $FeCl_3 \cdot K_3Fe(CN)_6$-,[2] magnesium acetate-,[3] and leuco-

*1 Melting point is uncorrected.

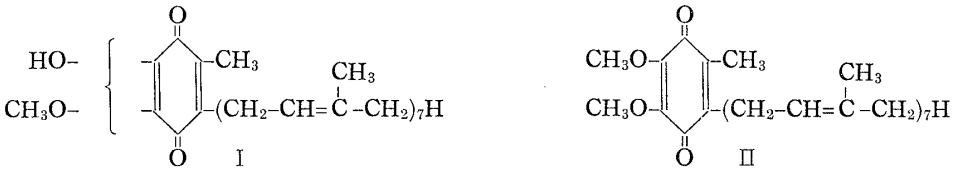
1) R.L. Lester, T. Ramasarma : J. Biol. Chem., **234**, 672 (1959).
2) G.M. Barton, R.S. Evans, J.A.F. Gardner : Nature, **170**, 249 (1952).
3) S. Shibata : Yakugaku Zasshi, **61**, 320 (1941).

Table II. Nuclear Magnetic Resonace Spectrum of Substance (I)

Proton type	τ[a]	Relative band areas	No. of protons based on $1CH_3O$/mole	No. of protons calcd. for $C_{43}H_{64}O_4$ and structure (I)
$\underline{H}C=$	4.9~5.0	217	7	7
$C\underline{H}_3O-$	6.0	92	3	3
$=C-C\underline{H}_2-CH=$	6.7~6.9	71	2	2
$=C-C\underline{H}_2-C\underline{H}_2-C=$	7.9~8.0	913	30	28
$C\underline{H}_3C=$ (nucleus)				
$O\underline{H}$				
$C\underline{H}_3C=$ (chain)	8.3~8.4	779	25	24

a) The bands refer to 60 Mc. spectra in carbon tetrachloride, using tetramethylsilane as an internal standard (Varian-A-60).

methylene blue-reaction.[4] When acetylated with acetic anhydride and pyridine, th substance gave an acetyl derivative which showed no absorption due to OH and exhibited a new absorption (CH_3CO) at 1790, 1190 cm^{-1} in infrared spectrum. From these results and the fact that the reduced form of Substance (I) was positive to neotetrazolium reaction,[1] Substance (I) was assumed to be demethyl-ubiquinone (35) (I).



The assumption was confirmed by methylation of Substance (I) to UQ (35) (II) with dimethylsulfate and anhydrous potassium carbonate. But it still remained unsolved which of the two methoxyl groups in UQ (35) was demethylated in the product (I). According to Lester, *et al.*[5] restoration of the activity of succinoxidase by Substance (I) was investigated using acetone-extracted mitochondria of beef-heart, and it was found that Substance (I) had a biological activity similar to that of UQ (35). Recently Glover, *et al.*[6] obtained a substance (Rhodoquinone) similar to Substance I from a photosynthetic bacterium, *Rhodospirillum rubrum*. It is interesting to note that both substances, Substance (I) and Rhodoquinone, were obtained by reactions relating with light.

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4) B.O. Linn, A.C. Page, Jr., E.L. Wong, P.H. Gale, C.H. Shunk, K. Folkers : J. Am. Chem. Soc., **81**, 4007 (1959).
5) R.L. Lester, S. Fleischer : Biochim. Biophys. Acta, **47**, 358 (1961).
6) J. Glover, D.R. Threlfall : Biochem. J., **85**, 14p (1962).